ELSEVIER

Postharvest Biology and Technology 13 (1998) 27–36

Postharvest Biology and Technology

Wounding, wound healing and staining of mature pear fruit

Robert A. Spotts [a,*], Peter G. Sanderson [b], Cheryl L. Lennox [a], David Sugar [c], Louis A. Cervantes [a]

[a] *Mid-Columbia Agricultural Research and Extension Center, Oregon State University, 3005 Experiment Station Drive, Hood River, OR 97031, USA*

[b] *Washington Tree Fruit Research Commission, 1719 Springwater Avenue, Wenatchee, WA 98801, USA*

[c] *Southern Oregon Research and Extension Center, 569 Hanley Road, Medford, OR 97502, USA*

Received 7 August 1997; accepted 13 November 1997

Abstract

Incidence of wounding in commercially-harvested 'd'Anjou' and 'Bosc' pear fruit, healing of wounds to decrease decay caused by *Botrytis cinerea*, *Mucor piriformis*, *Penicillium expansum*, and *Penicillium solitum* at −1°C, 20°C, and 28°C, and formation of compounds potentially involved in resistance were determined. Use of a blue food coloring to make wounds on fruit more visible on packinghouse lines was evaluated. Over 4 years, an average of 2.9% of 'd'Anjou' pear fruit were wounded during harvest and handling. In 'Bosc' pears, average incidence of wounding was 4.3% where fruit was harvested by workers paid by the hour, and 13.9% where workers were paid by the number of bins harvested. Susceptibility of wounds to infection by *M. piriformis*, *P. expansum*, and *B. cinerea* at −1°C decreased rapidly during the first 2 weeks, 4 weeks, and 8 weeks, respectively. The percent of wounds that stained well with food coloring was similar to the decay susceptibility curve for *P. expansum* at −1°C. Susceptibility of wounds to decay in fruit held at 20°C decreased almost linearly from 0 to 2 days. After 2 days at 20°C, 78% of the wounds absorbed stain, a significantly higher percent than were susceptible to decay. Prestorage heat treatment of fruit to 28°C for 24 h prior to inoculation decreased susceptibility of wounds to infection by both *P. expansum* and *P. solitum*. In commercial packinghouses, use of a 10% solution of blue food coloring to enhance visibility of wounds in pear fruit resulted in removal of 40% of punctured fruit compared with 22% removal without staining. Histochemical tests of cell walls near wounds showed an accumulation of callose, suberin, tannins and pectic substances, as well as gums and starch, within 4 days after wounding. Lignin was not detected in wound tissue.

Keywords: *Pyrus communis*; *Penicillium expansum*; *Penicillium solitum*; *Botrytis cinerea*; *Mucor piriformis*; Decay; Postharvest; Blue mold; Gray mold; Mucor rot

* Corresponding author.

0925-5214/98/$19.00
PII S0925-5214(97)00079-3

1. Introduction

Botrytis cinerea Pers.:Fr. (gray mold), *Mucor piriformis* Fischer (Mucor rot), and *Penicillium expansum* Link and *Penicillium solitum* Westling (blue mold) are major pathogens of pear fruit (Pierson et al., 1971; Sanderson and Spotts, 1995). In most cases, these fungi require a wound in the epidermis or stem to enter susceptible tissue and initiate infection. Infection occurs during harvest or as fruit move through contaminated water in the packingline dump tank or flumes (Spotts and Cervantes, 1986; Sugar and Spotts, 1993). Commercial pear packinglines include a sorting table where unacceptable fruit (culls) are removed for various reasons such as punctures, bruises, and decay. Wounded fruit that escape detection at the sorting table are placed into cold storage along with healthy fruit and may serve as foci for secondary spread of decay. Decay losses of winter pears in Washington and Oregon have been estimated at $2.5 million per year (E.M. Kupferman, Washington State University, pers. commun., 1993).

Wound healing in developing apple fruit has been largely associated with wound periderm formation, which is lacking in fruit wounded after harvest (Simons and Aubertin, 1959; Skene, 1981). Storage of 'Delicious' apples in a nonrefrigerated warehouse for 3 days prior to storage at 0°C reduced the susceptibility of lenticels to infection by *P. expansum* (English et al., 1946). Wounds in mature 'Golden Delicious' and 'Granny Smith' apples became resistant to *B. cinerea* and *P. expansum* within 4 days at 5°C (Lakshiminarayana et al., 1987). The fruit tissue exhibited formation of wall thickenings extending 4–6 cell layers from the wound. Cell walls near healed wounds stained positive for phenolic substances, tannins, lignins, and callose after 38 days at 5°C or 14 days at 20°C. In oranges and lemons, increased resistance to infection of injured peel is related to lignification of cells in the injured area (Brown, 1975; Baudoin and Eckert, 1982, 1985).

Wound healing of pear fruit has not been studied. However, prestorage treatments in which fruit were heated for 1, 3, and 5 days at 32°C, 27°C, and 21°C, respectively (Spotts and Chen, 1987), resulted in large decreases in side rot caused by *Phialophora malorum* (M.N. Kidd and A. Beaumont) McCulloch. In the same study, heating wounded fruit reduced Mucor and side rots, even when the fruit were inoculated after heating, indicating that heating may promote a wound healing response.

The objectives of this study were:

1. to determine the extent of wounding in commercially-harvested 'd'Anjou' and 'Bosc' pear fruit,
2. to study the relationship between wound healing and decay incidence caused by *B. cinerea*, *M. piriformis*, *P. expansum*, and *P. solitum* at −1, 20, and 28°C, and
3. to determine the presence and possible accumulation of compounds at or near wound sites that may be involved in resistance of wounds to decay.

In addition, we evaluated a food coloring for staining wounds at −1°C and 20°C and report the results of commercial trials with the food coloring to enhance visibility of wounded fruit during sorting.

2. Materials and methods

2.1. Survey of fruit for punctures during harvest

During commercial harvest of 'd'Anjou' pears with flesh firmness of 67 N (Hansen and Mellenthin, 1979) at the Mid-Columbia Agricultural Research and Extension Center, 100 fruit were arbitrarily removed from each of 12, 10, 9, and 8 wooden bins (454 kg of fruit) in 1992–1995, respectively, and the extent of wounding was determined. Fruit in each bin were harvested by a different person. Fruit were evaluated visually for breaks in the epidermis. The number of fruit with breaks was counted, and the longest diameter of each break was measured.

Wounding was similarly surveyed during harvest of 'Bosc' pears in commercial orchards in Medford, Oregon in 1994. Ten bins of fruit, each harvested by a different person, were examined in each of 15 orchards. One hundred fruit were examined from each bin, and the number and

longest diameter of wounds were recorded. In addition, 10 fruit collected from each bin were taken to the laboratory and tested for firmness with a Magness-Taylor penetrometer (Western Industrial Supply, San Francisco, CA).

2.2. Wound susceptibility to decay and staining

'd'Anjou' pear fruit were surface-sterilized with 100 μg sodium hypochlorite per ml, then rinsed with tap water. Each fruit was wounded at two locations with a metal tool that made a semicircular wound of 6 mm diameter and 3 mm depth. Fruit were placed at -1°C and 20°C in a cardboard fruit box lined with a perforated polyethylene bag. At 0, 1, 2, 3, 4, 8, and 12 weeks after wounding, fruit at -1°C were inoculated by placing 20 μl of spore suspension of *B. cinerea* (4000 conidia ml^{-1}), *M. piriformis* (2000 sporangiospores ml^{-1}), *P. expansum* (2000 conidia ml^{-1}), or sterile distilled water (SDW) in each wound. Fruit at 20°C also were similarly inoculated at 0, 1, and 2 days after wounding. Twelve replicate fruit were inoculated with each fungus at each time. After inoculation, fruit were incubated at 20°C in a cardboard fruit box lined with a perforated polyethylene bag. Lesion numbers and diameter were determined after 7 days. The experiment was performed three times.

As a result of preliminary studies in which various colors and concentrations of food coloring were tested to make wounds in pear fruit more visible, blue coloring was selected for further research. At each of the above inoculation times, wounds in 12 fruit were stained by placing 30 μl of a 10% solution of blue food coloring (blue flag liquid food color 47114, Crescent Foods, Inc., Seattle, WA) into each wound. The blue stain was removed with a fresh water rinse after 30 s. The degree of staining was evaluated visually on a scale of 1 to 3 where 1 = no staining, 2 = slight staining, and 3 = good staining.

2.3. Wound susceptibility – Prestorage heat and histology

'd'Anjou' pear fruit were surface-sterilized as above but without rinsing. Each fruit was wounded at two locations (6 mm diameter circle × 3 mm deep). Fruit were placed at -1°C and inoculated after 0, 1, 2, 4, 7, 14, and 30 days. Each wound received 50 μl of a conidial suspension of *P. expansum* or *P. solitum* containing 2×10^3 spores ml^{-1} or SDW (control). Fruit were incubated at -1°C for 16 weeks, then evaluated for decay incidence (percent of wounds infected) and severity (lesion diameter). After evaluation, decayed fruit were removed to prevent secondary spread. Remaining fruit were ripened at 20°C for 7 days and evaluated again for decay. Four replicates of five fruit were used for each time/pathogen combination.

In a second experiment, fruit were surface-sterilized, wounded, and inoculated as above either before or after heating to 28°C at 88 $\pm$ 4% relative humidity for 24 h. Control fruit were inoculated immediately after wounding and placed at -1°C without heating. After inoculation, heated fruit were incubated at -1°C for 6 weeks, then evaluated for decay incidence. Five replications of five fruit each were used for each heat treatment/pathogen combination.

In both experiments above, a separate set of wounded, noninoculated fruit were included in each treatment for histochemical tests. Fruit stored at -1°C were sampled at 0, 1, 2, 4, 7, 14 and 30 days after wounding, whereas fruit heated at 28°C were sampled after 0, 12 and 24 h of heating. Fruit samples were taken by excising tissue surrounding the wound with a scalpel. Excised tissue was vacuum infiltrated and fixed in formalin alcohol (70% ethanol) acetic acid (FAA) (Berlyn and Miksche, 1976), then dehydrated in three changes of tertiary butyl alcohol and embedded in Paraplast (Lancer, St. Louis, MO) using a standard wax embedding protocol (Jensen, 1962). The tissue was then sectioned on a rotary microtome set at 10 μm and heat-fixed to glass slides. Histochemical tests were carried out to determine the presence and possible accumulation of compounds at or near wound sites, and to determine whether heating of the fruit has an influence on the accumulation of these compounds. Aniline blue under ultraviolet light (Currier, 1957) and lacmoid (Ramming et al., 1973) were used to detect callose. Lignins were examined using

phloroglucinol and hydrochloric acid (Johansen, 1940), and chlorine-sulfite (Siegel, 1953). The fat soluble dyes Sudan IV (Rawlins and Takahashi, 1952) and Sudan Black B (Wade and Cruikshank, 1992) were used to stain for suberin. Reactions with ferric chloride (Rawlins and Takahashi, 1952), ferric sulfate (Reeve, 1959) and nitroso (Reeve, 1951) were used to detect tannins. Ruthenium red (Johansen, 1940) was used to detect pectic substances, and potassium iodide-iodine (Johansen, 1940) to detect starch. Mucicarmine with a fast green counterstain (Moore, 1965) was used to stain wound gums.

2.4. Commercial wound stain trials

At each of five commercial packinghouses in 1992 and three in 1993, 60 'd'Anjou' pear fruit were selected from fruit being packed at the time. One wound (6 mm diameter semicircle × 3 mm deep) was made in each fruit. Half of the fruit were immersed in a 10% solution (v/v) of blue food stain for 15 s, then rinsed with tap water. The other 30 fruit were immersed in tap water for 15 s, then rinsed. Stained and nonstained fruit alternately were placed, one fruit every 10 s, in the dump tank with the commercial fruit. Fruit moved through the packingline and flumes and across the sorting table where packinghouse personnel removed cull fruit that were punctured, decayed, or had other visible defects. Immediately prior to and after placing treated fruit in the tank, red rubber balls were placed in the tank as markers. Packinghouse personnel were instructed to direct the balls to the cull bin, along with culled fruit. All fruit culled between the two balls were examined visually, and the percent of stained and nonstained experimental fruit culled by the sorters was calculated at each packinghouse.

3. Results

3.1. Survey of fruit for punctures during harvest

An average 2.9% of 'd'Anjou' pear fruit were wounded during harvest. Average wound size was 5.3 mm (Table 1). Most wounds were caused by the stem of one fruit puncturing another fruit either in the picking bag or as fruit were transferred to bins. Occasionally, cuts from bin edges or from fingernails were observed. Payment of workers was based on the number of bins harvested.

Table 1
Wounded 'd'Anjou' pear fruit in bins at harvest, Mid-Columbia Agricultural Research and Extension Center, Hood River

Year	Wounded fruit (%)	Wound diameter (mm)
1992	4.0	5.7
1993	4.2	6.2
1994	1.3	4.7
1995	1.9	4.5
Avg. ± S.E.	2.9 ± 0.8	5.3 ± 0.4

In 'Bosc' pears surveyed in commercial orchards in Medford, Oregon, an average of 7.5% of the fruit were wounded during harvest. The average bin in orchards where workers were paid by the hour had 4.3% wounded fruit, while the average bin in orchards where workers were paid by the number of bins harvested had 13.9% wounded fruit (Table 2). The average wound diameters were 5.3 and 4.4 mm in each payment situation, respectively. Average fruit firmness values in surveyed orchards ranged from 55–68 N, but the relationship between wound incidence and fruit firmness among orchards was very weak ($R^2 = 0.16$).

Table 2
Wounded 'Bosc' pears in bins at harvest in commercial orchards with hourly and per bin payment of harvest labor (Medford, OR, 1994)

Method of paying harvest labor	Avg. % wounded fruit (± S.E.)	Wound diameter (mm) (± S.E.)
Hourly (10 orchards)	4.3 ± 1.4	5.3 ± 0.5
Per bin harvested (5 orchards)	13.9 ± 3.0	4.4 ± 0.8

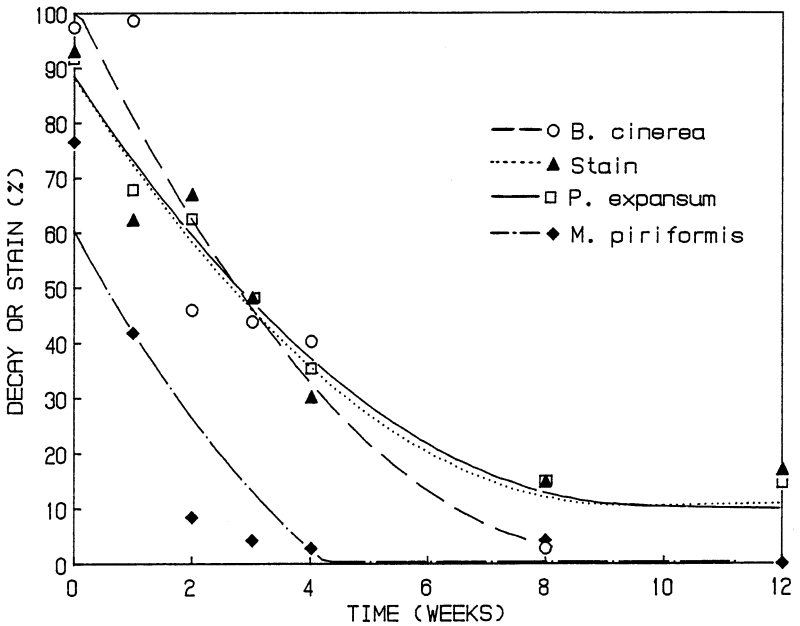

Fig. 1. Effect of time delay on infection or staining of wounds in 'd'Anjou' pear fruit held at −1°C for selected times before inoculation or staining. After inoculation, fruit were incubated at 20°C for 7 days, then evaluated. The quadratic equations for each pathogen or stain are: Percent decay (*B. cinerea*) = $102.0 - 22.2x + 1.2x^2$ ($R^2 = 0.902$*); Percent decay (*M. piriformis*) = $60.4 - 19.4x + 1.2x^2$ ($R^2 = 0.780$*); Percent decay (*P. expansum*) = $88.6 - 16.2x + 0.8x^2$ ($R^2 = 0.989$**); Percent stained = $88.4 - 16.8x + 0.9x^2$ ($R^2 = 0.952$**), where x represents time in weeks, * = significance at $P = 0.05$, and ** = significance at $P = 0.01$.

3.2. Wound susceptibility to decay and staining

Results of all three trials were similar, and data were combined for statistical analysis. Susceptibility of wounds in fruit to infection by *M. piriformis* and *B. cinerea* at −1°C decreased rapidly during the first 2 and 8 weeks, respectively, and little change occurred after these times (Fig. 1). For *P. expansum*, the percentage of fruit that decayed decreased from 93% to 35% by 4 weeks after wounding to 15% 8 weeks after wounding. No change in decay of fruit inoculated with *P. expansum* was observed after 8 weeks. No decay occurred in control fruit. The percent of wounds that stained well (easily visible) was similar to the decay susceptibility curve for *P. expansum* (Fig. 1), with 93% of the wounds staining well immediately after wounding, 30% staining 4 weeks after wounding, and 16% staining after 12 weeks.

Susceptibility of wounds in fruit held at 20°C decreased almost linearly from 0 to 2 days (Fig. 2). After 2 days at 20°C, only 11%, 19%, and 37% of the wounds were susceptible to *B. cinerea*, *M. piriformis*, and *P. expansum*, respectively. No decay occurred in control fruit. A significantly higher proportion of wounds stained after 2 days (78%) than decayed (Fig. 2).

3.3. Wound susceptibility – Prestorage heat and histology

At −1°C, wounds gradually became less susceptible to decay until 14 days after wounding (Fig. 3). From 14 to 30 days after wounding, little change was observed in susceptibility to either *P. expansum* or *P. solitum*. The relationship between susceptibility and time was described by quadratic equations, and all relationships were significant at $P = 0.05$ (Fig. 3). No decay occurred in wounds inoculated with SDW.

Lesion size of wounds infected with *P. expansum* decreased with time at −1°C and changed from an average of 47.3 mm at time 0 to 15.6 mm at 30 days. Size of lesions on fruit inoculated with

P. solitum averaged 11.0 mm diameter, and no change in severity with time was apparent.

Heat treatment of fruit prior to inoculation decreased susceptibility of wounds to infection by both *P. expansum* and *P. solitum* (Table 3). Heating inoculated fruit reduced decay caused by *P. solitum* but not *P. expansum* (Table 3).

Immediately after wounding, the wounded cell walls were thin and collapsed. With time, the outer walls compacted together, and formed a dense fibrous layer which lined the wound surface. An examination of cell morphology showed that cells adjacent to the wound surfaces had no meristematic activity. Starch grains were scattered in the tissue around the wound after wounding. However, with time, starch grains accumulated in the fibrous layer around the wound. Histochemical tests of cell walls near wounds showed that callose, suberin, tannins and pectic substances, as well as gums and starch accumulated in wounded pear tissue (Table 4). At 4 days after wounding, this fibrous barrier of cell walls was impregnated with suberin and tannin. Lignin was not detected in the wound tissue. Tests for callose, suberin and starch indicated that a 28°C heat treatment of 1 day accelerated the wound healing process, and that wounds from fruit placed immediately into storage at −1°C showed a delayed healing of 1 to 3 days when compared with wounds in heat treated fruit.

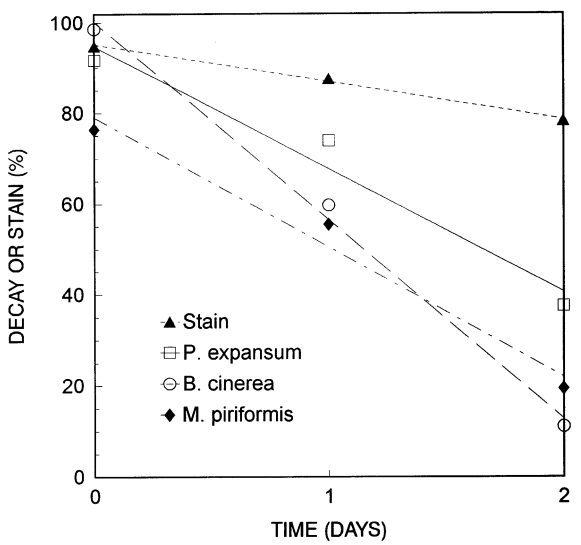

Fig. 2. Effect of time delay on infection or staining of wounds in 'd'Anjou' pear fruit held at 20°C for selected times before inoculation or staining. After inoculation, fruit were incubated at 20°C for 7 days, then evaluated. The linear regression equations for each pathogen or stain are: Percent decay (*B. cinerea*) = $100.2 - 43.7x$ ($R = -0.998$*); Percent decay (*M. piriformis*) = $78.9 - 28.5x$ ($R = -0.988$); Percent decay (*P. expansum*) = $94.8 - 27.1x$ ($R = -0.981$); Percent stained = $95.2 - 8.3x$ ($R = -0.998$*), where x represents time in weeks, * = significance at $P = 0.05$.

3.4. Commercial wound stain trials

A greater percent of stained fruit than nonstained was recovered at seven of the eight packinghouses (Table 5), and the effect of staining on recovery of wounded fruit was significant ($P = 0.028$). Overall, 39.6% of stained fruit and 21.7% of nonstained fruit were detected and removed as cull fruit by packinghouse personnel.

4. Discussion

Almost 3% of 'd'Anjou' pear fruit were wounded during harvest. If these fruit were exposed to *B. cinerea* and *P. expansum* at spore concentrations typically found in packinghouse dump tank and flume water (Spotts and Cervantes, 1986; Sanderson and Spotts, 1995), about a third (1% of the fruit) would become infected (Spotts, 1986). These primary infections, when accompanied by later-developing secondary spread and stem end and calyx end decay, could constitute a serious commercial problem.

The two to three times higher incidence of wounding in 'Bosc' than in 'd'Anjou' pears may reflect cultivar differences in susceptibility due to fruit shape (Sugar and Penwell, 1989). 'd'Anjou' fruit were harvested at a similar level of maturity each year based on flesh firmness, and the greater extent of wounded fruit in 1992–1993 compared with 1994–1995 may be due to less care of people harvesting the fruit in the first two years of the study. Greater wounding of 'Bosc' pears in orchards harvested by workers paid by the number of bins harvested than in orchards where workers were paid by the hour may be due to differences

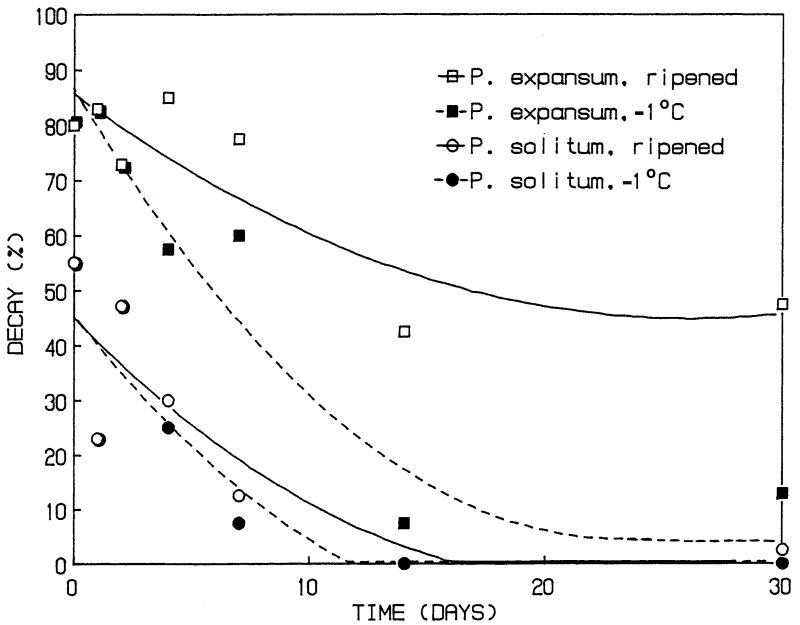

Fig. 3. Effect of time delay on infection of wounded 'd'Anjou' pear fruit held at −1°C for selected times before inoculation. After inoculation, fruit were incubated at −1°C for 16 weeks, then evaluated (solid symbols) or ripened at 20°C an additional 7 days (open symbols) before evaluation. The quadratic equations for each pathogen are: Percent decay (*P. expansum*, −1°C for 16 weeks + 20°C for 7 days) = $85.7 - 3.1x + 0.1x^2$ ($R^2 = 0.759$*); Percent decay (*P. expansum*, −1°C for 16 weeks) = $86.7 - 7.1x + 0.2x^2$ ($R^2 = 0.930$**); Percent decay (*P. solitum*, −1°C for 16 weeks +20°C for 7 days) = $45.1 - 4.4x + 0.1x^2$ ($R^2 = 0.754$*); Percent decay (*P. solitum*, −1°C for 16 weeks) = $45.6 - 5.4x + 0.1x^2$ ($R^2 = 0.796$*), where x represents time in weeks, * = significance at $P = 0.05$, and ** = significance at $P = 0.01$.

in care in handling the fruit. Encouraging workers to harvest faster by paying by the number of bins harvested may be counter-productive for growers, especially with the wound-prone 'Bosc' cultivar.

The susceptibility of wounds to blue mold in fruit held at −1, 20, and 28°C decreased significantly after 4 weeks, 2 days, and 1 day, respectively. When fruit were returned to −1°C after inoculation rather than immediately ripened at 20°C, susceptibility was lowest after only 2 weeks at −1°C prior to inoculation. At the above temperature/time combinations, resistance to Mucor rot, gray mold, and blue mold caused by *P. solitum* was usually even greater than to *P. expansum*. These changes in wound susceptibility are similar to those previously reported for apple fruit (English et al., 1946; Lakshiminarayana et al., 1987). *Penicillium expansum* is more virulent than *P. solitum* (Sanderson and Spotts, 1995), and this study confirmed the previous report.

Use of blue food coloring to enhance visibility of wounds resulted in removal of 40% of punctured fruit compared with 22% removal without staining. This technique should be a useful component of an integrated decay control program. In addition, there appeared to be a close relationship between incidence of blue mold and staining of wounds. The percent of wounds in fruit held at −1°C and 20°C that stained also was similar to the percent of wounds susceptible to decay by *B. cinerea* and exceeded the percent susceptible to *M. piriformis*. Thus, removal of fruit with stained wounds would likely result in removal of a high proportion of wounded fruit susceptible to infection by the three major postharvest decay fungi of pear. Further studies in commercial packinghouses are necessary to validate the food coloring technique for reduction of decay.

The formation of impervious tissue is the most common feature observed in the wound responses

Table 3
Effect of prestorage heat treatment on decay of 'd'Anjou' pear fruits

Treatment sequence[a]	Percent decay[b] caused by	
	Penicillium expansum	*Penicillium solitum*
Inoculate (no heat)	80b	55b
Inoculate, then heat	100c	2a
Heat, then inoculate	0a	0a

[a] Fruits heated at 28°C for one day, then evaluated after storage at −1°C for 6 weeks.
[b] Each value represents the average of five replications of five fruits per replication, each fruit wounded twice. Numbers followed by the same letter are not significantly different at $P = 0.01$ according to protected least significant difference (LSD) test.

Table 5
Effect of wound staining on recovery of punctured 'd'Anjou' pear fruit on sorting tables

Packing house[a]	Percent fruit recovered[b]	
	Stained	Nonstained
A	40.0	10.0
B	56.7	60.0
C	6.7	3.3
D	40.0	36.7
E	3.3	0.0
F	73.3	26.7
G	46.7	20.0
H	50.0	16.7

[a] Packinghouses trials A–E performed in 1992, F–H in 1993.

[b] Effect of staining significant at $P = 0.0284$ according to paired t-test.

of herbaceous and woody plants (Bostock and Stermer, 1989). In this study, the dense fibrous layer of cells which lined the wounds may provide a barrier to infection by pathogenic organisms and prevent water loss from the underlying cells.

The nonmeristematic wound response of mature pear fruit observed in this study is similar to that found in apple fruit where immature but not mature apple fruit form wound periderm (Simons and Aubertin, 1959; Skene, 1981). Wound healing in mature apple fruit appears to involve cell wall thickening and formation of phenolic substances,

Table 4
Histochemical tests for presence and accumulation of compounds in wound tissue of 'd'Anjou' pear fruit at selected time intervals after wounding

Compound	Test	Reaction in fruit stored at −1°C[a] Time after wounding (days)							Reaction in fruit heated at 28°C[a] Time after wounding (h)		
		0	1	2	4	7	14	30	0	12	24
Callose	Analine blue	+	+	++	+	++	++	++	+	+	++
	Lacmoid	−	−	−	−	−	−	−	−	−	−
	Cotton blue	−	−	−	−	−	−	−	−	−	−
Gums	Mucicarmine	+	++	++	++	++	++	++	+	+	++
Lignin	Phloroglucinol	−	−	−	−	−	−	−	−	−	−
	Chlorine sulphite	−	−	−	−	−	−	−	−	−	−
Pectic substances	Ruthenium red	+	+	+	++	++	++	++	+	++	++
Starch	Iodine	+	+	+	++	++	+ +	++	+	+	++
Suberin	Sudan IV	−	+	+	++	++	++	++	−	+	++
	Sudan black B	−	+	+	++	++	+ +	++	−	+	++
Tannin	Ferric chloride	−	−	−	−	−	−	−	−	−	−
	Ferric sulphate	−	−	−	−	−	−	−	−	−	−
	Nitroso reaction	+	++	++	++	++	++	+ +	−	++	++

[a] Reactions designations as follows: −, compound not detected; +, present; ++, present in greater quantity than +.

tannins, lignin, and callose (Lakshiminarayana et al., 1987). These compounds have been associated with wound healing in many plants and have been implicated in resistance to infection and colonization in many host/pathogen systems (Bostock and Stermer, 1989). The wounded pear tissue rapidly accumulated callose and tannins, as well as gums; but tests for lignin were negative. It is possible that this study did not allow sufficient time for lignin to accumulate in the wound tissue or that lignin was lost during fixation of the tissue in FAA. Compounds thought to be involved in wound healing were detected in apple tissue after 38 days at 5°C followed by 14 days at 20°C but analyses were not carried out at shorter times (Lakshiminarayana et al., 1987). In our study, the prestorage heat treatment of one day at 28°C appears to have accelerated the wound healing process by 12 h (tannin), 24 h (callose), and 72 h (pectic substances, starch, and suberin). Thus, the accumulation of these compounds appears to precede or coincide with the increase in resistance to decay. Although additional research is necessary to prove a causal relationship, the positive correlation implicates several of these compounds as part of the complex mechanisms involved in wound healing in mature pear fruit.

Acknowledgements

This project was supported in part by the Winter Pear Control Committee. Use of trade names in this article does not imply endorsement by Oregon State University of the products named or criticism of similar products not mentioned. Oregon Agricultural Experiment Station Technical Paper 11 218.

References

Baudoin, A.B.A.M., Eckert, J.W., 1982. Factors influencing the susceptibility of lemons to infections by *Geotrichum candidum*. Phytopathology 72, 1592–1597.

Baudoin, A.B.A.M., Eckert, J.W., 1985. Development of resistance against *Geotrichum candidum* in lemon peel injuries. Phytopathology 75, 174–179.

Berlyn, G.P., Miksche, J.P., 1976. Botanical Microtechnique and Cytochemistry. Iowa State University Press, Ames, Iowa.

Bostock, R.M., Stermer, B.A., 1989. Perspectives on wound healing in resistance to pathogens. Annu. Rev. Phytopathol. 7, 343–371.

Brown, G.E., 1975. Factors affecting postharvest development of *Colletotrichum gloeosporioides* in citrus fruits. Phytopathology 65, 404–409.

Currier, H.B., 1957. Callose substance in plant cells. Amer. J. Bot. 44, 478–488.

English, H., Ryall, A.L., Smith, E., 1946. Blue Mold Decay of Delicious Apples in Relation to Handling Practices. Circular 751, US Department of Agriculture, Washington, DC, 19 pp.

Hansen, E., Mellenthin, W.M., 1979. Commercial handling and storage practices for winter pears. Agric. Exp. Stn., Oreg. State Univ., Corvallis, Oreg., Spec. Rept., 550.

Jensen, W.A., 1962. Botanical Histochemistry. W.H. Freeman and Co., San Francisco.

Johansen, D.A., 1940. Plant Microtechnique. McGraw-Hill Co., New York.

Lakshiminarayana, S., Sommer, N.F., Polito, V., Fortlage, R.J., 1987. Development of resistance to infection by *Botrytis cinerea* and *Penicillium expansum* in wounds of mature apple fruits. Phytopathology 77, 1674–1678.

Moore, E.J., 1965. Staining fungal gel with mucin techniques. Stain Technology 40, 23–27.

Pierson, C.F., Ceponis, M.J., McColloch, L.P., 1971. Market diseases of apples, pears, and quinces. Agricultural Handbook 376, U.S. Department of Agriculture, Washington, DC, 112 pp.

Ramming, D.W., Hinrichs, H.A., Richardson, P.E., 1973. Sequential staining of callose by aniline blue and lacmoid for fluorescence and regular microscopy on a durable preparation of the same specimen. Stain Tech. 48, 133–134.

Rawlins, T.E., Takahashi, W.N., 1952. Techniques of Plant Histochemistry and Virology. National Press, Millbrae, California.

Reeve, R.M., 1951. Histochemical tests for polyphenols in plant tissues. Stain Tech. 26, 91–96.

Reeve, R.M., 1959. Histological and histochemical changes in developing and ripening peaches. I. The catechol tannins. Am. J. Botany 46, 210–217.

Sanderson, P.G., Spotts, R.A., 1995. Postharvest decay of winter pear and apple fruit caused by species of *Penicillium*. Phytopathology 85, 103–110.

Siegel, S.M., 1953. On the biosynthesis of lignin. Physiol. Plantarum 6, 134–139.

Simons, R.K., Aubertin, M.P., 1959. Development of epidermal, hypodermal, and cortical tissues in the 'Golden Delicious' apple as influenced by induced mechanical injury. Proc. Amer. Soc. Hortic. Sci. 74, 1–9.

Skene, D.S., 1981. Wound healing in apple fruits: The anatomical response of 'Cox's Orange Pippin' at different stages of development. J. Hortic. Sci. 56, 145–153.

Spotts, R.A., 1986. Relationships between inoculum concentrations of three decay fungi and pear fruit decay. Plant Dis. 70, 386–389.

Spotts, R.A., Cervantes, L.A., 1986. Populations, pathogenicity, and benomyl resistance of *Botrytis* spp., *Penicillium* spp., and *Mucor piriformis* in packinghouses. Plant Dis. 70, 106–108.

Spotts, R.A., Chen, P.M., 1987. Prestorage heat treatment for control of decay of pear fruit. Phytopathology 77, 1578–1582.

Sugar, D., Penwell, M., 1989. Packing 'Bosc' Pears. Proc. 5th Ann. Warehouse Seminar. Wash. State Hort. Assoc., Wenatchee, pp. 22-23.

Sugar, D., Spotts, R.A., 1993. The importance of wounds in infection of pear fruit by *Phialophora malorum* and the role of hydrostatic pressure in spore penetration of wounds. Phytopathology 83, 1083–1086.

Wade, G.C., Cruikshank, R.H., 1992. Rapid development of resistance of wounds on immature apricot fruit to infection with *Monilinia fructicola*.. J. Phytopath. 136, 89–94.